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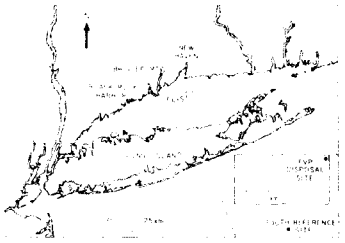

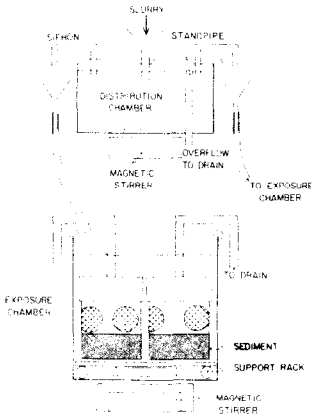

US Army Corps
of Engineers

FIELD VERIFICATION PROGRAM
(AQUATIC DISPOSAL)

TECHNICAL REPORT D-86-1

HISTOPATHOLOGICAL EFFECTS OF BLACK ROCK HARBOR DREDGED MATERIAL ON MARINE ORGANISMS

A LABORATORY INVESTIGATION

by

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Final Report

Prepared for DEPARTMENT OF THE ARMY
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The D-series of reports includes publications of the Environmental Effects of Dredging Programs:

Dredging Operations Technical Support

Long-Term Effects of Dredging Operations

Interagency Field Verification of Methodologies for Evaluating Dredged Material Disposal Alternatives (Field Verification Program)

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Histopathological Effects of Black Rock Harbor Dredged Material on Marine Organisms; A Laboratory Investigation"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of a generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Histopathological Effects of Black Rock Harbor Dredged Material on Marine Organisms; A Laboratory Investigation"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation are being conducted by WES and studies of aquatic disposal are being carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies are funded by the Corps while salary, support facilities, etc., are provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and will be published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

James Choromokos, Jr., Ph.D., P.E.
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U. S. Army Corps of Engineers

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Assistant Administrator for
Research and Development
U. S. Environmental Protection
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Histopathology studies were conducted on tissues representing the major organ systems of several marine organisms exposed in the laboratory to Black Rock Harbor dredged material. These studies examined the applicability of these procedures for measuring biological effects of dredged material and the variability and reproducibility of the methodology.

Female reproductive tract changes in the filter feeding bivalve mollusc, Mytilus edulis, included degeneration of ova and loss of nuclei, cytoplasm,

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and vitelline membrane. Cardiovascular changes in this species were characterized by pedunculated growths from the auricle, ventricle, and pericardial wall. Examination of the deposit feeding bivalve mollusc, Yoldia limatula, revealed no histological changes.

There was a well-defined pattern of histological changes in the amphipod, Ampelisca abdita, characterized by necrosis of gill epithelium and lamellae, loss of normal gill architecture, and changes in the mucous tube glands. The pathologies occurred consistently regardless of the route of contaminant exposure. The reproducibility of both types of responses was excellent.

The polychaete annelids, Nephtys incisa and Neanthes arenaceodentata, developed histopathological changes in the parapodial musculature, metaplasia of the epidermis, and degeneration of mucous cells (Neanthes). The responses were very consistent between replicated experiments both qualitatively and quantitatively. It is important to note that in both species, Neanthes and Ampelisca, where mucous cells were present, a similar pathological response was observed. The impairment of mucous cell production in these species has serious ecological implications.

The results from these studies indicate that histological changes can be used to measure the effects of dredged material and that the methodology, while inherently subjective, is reproducible when done by the same investigator. The potential problem with reproducibility of this technique lies with differences in the subjective nature of interpretation between different investigators,

PREFACE

This report describes work performed by the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Narragansett, R.I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). This program is sponsored by the Office, Chief of Engineers (OCE), and administered by the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi. The program objective of this interagency agreement is to verify existing predictive techniques for evaluating the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP study is being conducted by ERLN, with the wetland and upland portions done by WES.

The principal investigators for this aquatic study were Mr. Paul P. Yevich, Ms. Carolyn A. Yevich, Dr. K. John Scott, Ms. Michele Redmond, Dr. Paul S. Schauer, Ms. Carol Pesch, and Ms. Dianne Black. The slide preparation was done by Ms. Esther Peters and Mr. Michael Casey. The EPA Technical Director for the FVP was Dr. John H. Gentile, Technical Coordinator was Mr. Walter Galloway, and Project Manager was Mr. Allen Beck.

This study was conducted under the direct supervision of Dr. Richard K. Peddicord and Dr. Tom Dillon, Environmental Laboratory (EL); and under the general supervision of Dr. C. Richard Lee, Chief, Contaminant Mobility and Regulatory Criteria Group; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. John Harrison,

Chief, EL. Dr. Robert M. Engler was Manager, Environmental Effects of Dredging Program. Editorial review was performed by Ms. Jamie W. Leach of the WES Publications and Graphic Arts Division.

The OCE Technical Monitors were Drs. John Hall, Robert J. Pierce, and William L. Klesch. The Water Resources Support Center Technical Monitor was Mr. Charles W. Hummer.

Director of WES was COL Allen F. Grum, USA. Technical Director was Dr. Robert W. Whalin.

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PART I: INTRODUCTION

Background

1. Bioassay experiments are traditionally done both in the laboratory and in the natural environment to determine the effects of pollutants on plants or animals. These studies can be done at various levels of biological organization including ecosystem, population, and individual. Adverse impacts of pollutants at the ecosystem or population level result from the effects of the materials upon the individuals comprising a community group. Although the effects at higher levels of organization may appear more complex or subtle than those observed in individuals, tests on individuals can provide a basis for understanding community responses.

2. At the individual species level, tests are conducted to measure the effects of pollutants on the behavior, physiology, metabolism, growth, mortality, and reproduction of the organisms. Adverse effects of pollutants on growth, survival, and reproduction occur at the tissue and cellular levels. Histopathological examination via the light microscope can elucidate these effects on aquatic animals as it has with studies on humans and other mammals. Histopathological examination is an important tool in clinical, forensic, human, and veterinary medicine.

3. Histopathologic examinations are carried out to evaluate the health of an organism on the basis of the appearance of the cells and tissues. The degree of divergence from the normal cell structure indicates the relative degree of injury to the animals. When abnormal histological conditions are observed, an attempt is made to determine if these conditions are detrimental to the organism, if they are reversible or irreversible, and their cause. Changes may interfere with normal physiological functions, reproductive capability, and/or with the ability of the animal to survive. To recognize the subtle changes caused by pollutants, the histopathologist must know the difference between the normal histophysiological changes that occur during the life cycles of aquatic organisms, and those caused by harmful chemical, biological, and physical agents; disease; and parasites.

4. Information on "total body" and individual organ burdens of various metals and other chemicals is becoming increasingly important in controlling and monitoring toxic substances. Histopathologic examination enables one to determine if a particular compound has an adverse effect on an organism at sublethal concentrations or if the animal can accumulate large amounts of a pollutant without being harmed.

5. All organisms have mechanisms which, until they are overloaded, can detoxify compounds so that they will not injure the body, but the methods and capacity to detoxify vary among the species. Some invertebrates are especially adapted for concentrating certain metals. A good example of an organism that accumulates a toxicant without adverse histological effects is the oyster. It can accumulate copper until it is a deep green color. However, even in this condition, there

is no interference with the metabolism, growth, or reproduction of the animal. This is possible because the copper is taken up from the serum by the blood cells (amebocytes) and sealed off in membrane-limited vesicles (George et al., 1978). The copper concentration in these cells may be as high as 13,000 ppm. The animal is able to depurate these copper-containing blood cells into the water. Thus, when the level of copper is reduced in the environment, the animal's body burden will return to normal without tissue damage.

6. Barszcz et al. (1978) examined oysters (Crassostrea virginica) exposed to Empire Mix, Saudi Arabian, and Nigerian crude oil in estuarine ponds at a concentration of 4 ppm for a period of 9 months. Several detrimental histopathological effects were observed. There was a loss of tubules in the digestive diverticula, which indicated that the animals were not feeding. A degenerating condition of the muscle bundles and connective tissue, called hyaline degeneration, was noted along the mantle and the food groove of the gills. This condition indicated that the oil probably interfered with the protein metabolism of the cells. Also, in many animals there was a complete lack of development of the reproductive tract, which interfered with reproduction. The degree of severity of the above lesions varied with the type of crude oil to which the animals were exposed.

7. Gonzales et al. (1976) investigated the cause of death of large numbers of blue mussels, Mytilus edulis, in an effluent canal of a steam-powered electric generating plant. Histopathologic examination of surviving animals indicated that they are not feeding because of the loss of the frontal and lateral frontal cilia from the gill filaments.

These cilia are necessary for sorting out the food particles and moving them to the mouth. Ulcerations of the epithelium of the stomach and necrosis of the epithelium of the intestine were also observed. Thus, histopathologic examination showed that the elevated water temperature or toxic substances which may have been in the water resulted in loss of gill cilia and necrosis of the epithelium of the gastrointestinal tract, which eventually led to the death of the animals. The same pathology was observed in animals exposed in the laboratory only to temperature excursions patterned after the conditions at the power plant.

8. In a five-year study of a JP5 and #2 fuel oil spill site in Long Cove, Searsport, Maine, Yevich and Barszcz (1977) observed a 1-22% incidence of gonadal neoplasms (cancer) in the soft shell clams, Mya arenaria, collected from various stations in the cove. Gonadal tumors were not detected in M. arenaria collected from control sites. Because gonadal neoplasms could not be induced in the laboratory, a direct causal link between the oil and carcinogenesis could not be established. While there are many short-term tests for mutagens, such as the Ames test, the only way to determine if a substance is carcinogenic to a particular species is to expose the animals to the substance and look for histopathological changes in the tissues.

9. When carrying out acute toxicity tests, histopathological examination can be used to determine which tissues of the body are affected by the toxicant and elucidate the possible cause of death. A good example of this is provided by a study by Gardner et al. (1970). In this study, Fudulus heteroclitus, the mummichog, was exposed to cadmium at a concentration of 50 ppm, and the animals were collected

for examination at various time intervals during the exposure period. The following sequence of histopathologic effects was noted. After one hour of exposure, there was extensive necrosis of the intestinal mucosa. Necrosis of the uriniferous tubules occurred after 11 hours of exposure. The gill filaments showed histopathologic changes after 20 hours of exposure. Thus, the sequence in which the tissues were affected was (1) the epithelium of the intestine, (2) the kidneys, and (3) the gills. The discovery that the intestine was the first site of pathology was important because many researchers believed that when fish were exposed to a toxic metal, the first site of toxic action was always the gills.

10. In studies conducted at the Environmental Research Laboratory, Narragansett, fish and invertebrates (in particular, silversides, Menidia menidia, and mummichogs, Fundulus heteroclitus), when exposed to low concentrations of oils and metals, sometimes displayed unusual behavior patterns. No morphological alterations were noted in any of the visceral organs or tissues of these organisms, but histopathologic changes were observed in the neuroepithelium of the olfactory tract and neuromasts of the lateral line, providing a possible explanation for unusual behavior patterns.

Purpose

11. The study reported herein was conducted to determine the applicability of using histopathology to assess the effects of dredged material on epibenthic and infaunal marine organisms. Specifically, this report involves the histopathologic examination of Mytilus edulis

(mussels), Ampelisca abdita (amphipod), Nephtys incisa (polychaete), Neanthes arenaceodentata (polychaete), and Yoldia limatula (clam), all of which were exposed to various concentrations of Black Rock Harbor sediment in the laboratory. The objectives of this study are to determine first the applicability and sensitivity of using histopathological responses to measure impacts and second to determine the reproducibility of the responses. Unlike other objectively measured responses, the determination and interpretation of histopathological changes are subjective and dependent on the types of fixatives, staining procedures, and experience of the investigator. In addition, because histopathological data are often binomially distributed, parametric statistical analysis is not appropriate.

PART II: METHODS AND MATERIALS

Overview

12. The types of tests conducted to provide animals for histopathological study included both suspended particulate and solid phase exposures to Black Rock Harbor (BRH) sediments. Suspensions of either Reference (REF) or BRH sediment were dosed in various combinations with a solid phase ranging from 100 percent REF to 100 percent BRH sediment where appropriate. Tests combining the solid and particulate phase were representative of the type of condition at the disposal site; however, the concentrations of suspended material used in the tests did not necessarily simulate actual field concentrations. Concentrations were chosen to produce a dose response in the endpoint measurements.

13. The tests described above generally follow methods prescribed in "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians," American Society for Testing Materials (ASTM 1980). Although the ASTM test methods were not specifically designed for sediment tests, they provide guidelines for experimental designs, water quality parameters, statistical analyses, and animal care, handling, and acclimation.

Sediment Collection and Preservation

14. Reference sediment for these studies was collected from the Field Verification Program South reference site (40°7.95"N and 72°52.7"W), which is approximately 700 m south of the southern perimeter of the

Central Long Island Sound (CLIS) disposal site. Reference sediment was collected with a Smith-McIntrye grab sampler (0.1 m^2) in August and December 1982 and May 1983 (collections I, II, and III, respectively). Sediment from each collection was returned to the laboratory, press sieved (wet) through a 2-mm mesh stainless steel screen, homogenized and stored in polypropylene (collection I) or glass (collections II and III) containers at 4°C until used in experiments. Each container of material was coded with collection number, date, and jar number.*

15. Black Rock Harbor sediment was collected from 25 locations within the Black Rock Harbor (Bridgeport, Conn.) study area with a 0.1-m^2 gravity box corer to a depth of 1.21 m. The sediment was homogenized, distributed to barrels, and stored at 4°C. The contents of each barrel were homogenized, wet sieved through a 1-mm sieve, distributed to glass jars, and stored at 4°C until used in experiments. Samples of sediment were taken at various points during the collection, mixing, and distribution process for moisture content and chemical analysis.

General Histological Methods

16. Standard histological methods for aquatic animals will be available in the U.S. EPA manual, "Biological Field and Laboratory Methods for Measuring the Quality of Surface Water and Effluents" (Yevich and Barszcz, 1981) and in "The International Mussel Watch" (NAS, 1980). The following is a condensation of those methods and procedures applied in these studies.

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* See Rogerson, Schimmel, and Hoffman (1985) for complete details.

17. While preparing the animals for fixation, detailed observations of the appearance of the organisms were made and information on the date, species, area of collection, type of study, and other pertinent data, was recorded. Each animal was given a unique identification number. Fixation is the most important step in the preparation of aquatic animals for histopathological examination. Fixation is necessary to prevent autolysis (tissue self-destruction) and to preserve the tissue in as life-like condition as possible. Adequate fixation with the proper fixative is critical for the preservation of the cellular inclusions and secretions as well as the normal cell structure of aquatic animals.

18. Helly's fixative (Jones, 1964) made with zinc chloride instead of mercuric chloride is the fixative of choice for most invertebrates (Barszcz and Yevich, 1975). This is an excellent cytological fixative for aquatic invertebrates, preserving the nucleoplasm of the nuclei of ova, the granules of the secretory cells, and the amebocytes better than other fixatives tested. Tissues fixed in Helly's will exhibit an intense stain, with hematoxylin and eosin and many other special stains. However, the amount of time that the tissue remains in this fixative must be carefully controlled because prolonged fixation will cause excessive hardening and brittleness, making sectioning difficult or impossible. Fixation time for Helly's is dependent on the size and the density of the material. While twenty-four hours is usually considered the maximum allowable time, Table 1 gives suggested fixation times for various species of marine animals.

Table 1

Suggested Time in Fixatives

Species	Helly's[1]	Dietrich's[2]
Zooplankton	not recommended	2+ hr.
Anemones	2-16 hr.	2+ hr.
Worms	15 min. - 16 hr.	4+ hr.
Bivalves	2 - 16 hr.	8+ hr.
Gastropods	4 - 16 hr.	8+ hr.
Shrimp	4 - 16 hr.	8+ hr.
Fish	not recommended	8 - 24 hr.

1. The amount of time in Helly's fixative is dependent on the size and density of the tissue.

2. Time given is minimum amount. Tissues may be left in Dietrich's indefinitely.

Formula for Helly's Fixative

1,000 gm zinc chloride*
500 gm potassium dichromate
20 l distilled water
Add 5 ml of 37-40% formaldehyde per 100 ml of fixative at time of use

*Zinc chloride is used in Helly's fixative instead of mercuric chloride because it is less toxic and does not form a precipitate that must be removed during staining. It has the same fixing and mordanting qualities as mercuric chloride.

Formula for Dietrich's Fixative

9,000 ml distilled water
4,500 ml 95% ethanol
1,500 ml 40% formaldehyde
300 ml glacial acetic acid

19. Dietrich's fixative (Gray, 1954) is used for fish, amphipods, and other small zooplankton. It gives good cellular detail and there is little shrinkage or distortion of the cells. Animals can be stored in Dietrich's for 2-3 months if necessary without excessive hardening of the tissues. The major drawback to using Dietrich's or any other fixative that contains glacial acetic acid for invertebrates is that it causes the loss of secretions and granules and loss of nucleoplasm from ova.

20. Animals were placed in fixative while alive and allowed to fix for 15-40 minutes (to firm the tissues), removed, sectioned (e.g., sagittally, transverse, etc.), and returned to fixative. At the completion of fixation, the tissues were given a final trimming as necessary, washed to remove excess fixative, and then stored in S-29 dehydrating reagent. To prepare the tissues for penetration by paraffin, they were dehydrated in S-29 and cleared in UC-670, Technician's dehydrating and clearing reagents, on the Autotechnicon tissue processor. Following paraffin infiltration, the tissues were embedded into paraffin blocks. Tissue sections were cut at 6 microns on a rotary microtome, mounted on slides, and stained with Harris hematoxylin and eosin. Table 2 shows the amount of time necessary for each of the major steps involved in tissue processing (Bayne et al., 1980; Barszcz and Yevich, 1976; Yevich and Barszcz, 1981). All blocks were saved and recut as necessary to confirm observed abnormalities.

Table 2

General Procedures for Processing Tissues

Procedure	Time Necessary
Initial fixation	20 - 30 minutes
Initial trimming	as required
Final fixation	2 - 24 hours
Decalcification of bone or exoskeleton	Determine empirically minimum amount necessary
Final trimming	as required
Washing in water	16 - 24 hours
Storage of tissues	until processed
Dehydration, clearing and infiltration	4 - 16 hours
Embedding	as necessary
Sectioning	as required
Staining	as required

Mytilus edulis

Collection and holding

21. Mussels for each of three experiments were collected with a scallop dredge from an uncontaminated site near Dutch Island in the west passage of Narragansett Bay (71°24.0'W by 41°29.4'N) from depths ranging between 5 and 10 m. Collection information for each experiment is listed below:

Experiment	Collection Date	Experiment Begun	Field Temperature °C	Field Salinity °/oo
NOEC	10/7/83	10/11/83	17.5	31.0
Experiment A	11/10/83	11/16/83	13.0	31.0
Experiment B	3/8/84	3/19/84	5.0	29.0

The animals were sorted to obtain a size range of 50 to 55 mm shell length and held in a laboratory flow-through system in unfiltered seawater at a salinity equal to the field salinity until the experiment was initiated. All experiments were run at 15°C. Mussels collected from the field when the temperature was below 15°C were acclimated in running unfiltered seawater at a rate of 1°C per day until 15°C was reached.

Exposure methods

22. The laboratory documentation phase of the FVP for M. edulis consisted of three experiments. The first experiment was conducted because it was necessary to determine the level of uncontaminated suspended particulates that did not adversely affect the test animal. This determination was termed the no-observable-effect-concentration experiment (NOEC). This experiment is detailed in Appendix A of Nelson et al. (1985). The objective was to select the maximum exposure concentration of the reference suspended sediment which resulted in no significant reduction in scope for growth (SFG). The approach taken in the NOEC experiment was to expose mussels to different particulate concentrations (0, 6.25, 12.5, 25, 50, and 100 mg/ℓ) of suspended reference sediment. The results indicated that mussels in the 50-mg/ℓ treatment produced pseudofeces throughout the 28-day experiment, while those in the

lower treatment levels did not. This would maximize processing of, and thus exposure to, the suspended material through the gastrointestinal tract of the animal. In addition, mussels from this treatment also exhibited the highest SFG values. Based upon these data, 50 mg/ℓ was chosen as the total suspended solids concentration for subsequent experiments with BRH sediment.

23. Two experiments were designed to examine the effects of BRH suspended sediment on M. edulis. The exposure system is shown in Figure 1. In these experiments the REF and BRH mixing and distribution chambers (Figure 1) were maintained at 50 mg/ℓ. Exposure conditions were obtained by siphoning suspended sediment from the appropriate distribution chambers to produce a combined flow of 300 ml/min in each exposure chamber. The amount of suspended particulates both entering the exposure chambers (actual incoming concentration) and the concentration surrounding the mussels (actual surrounding concentration) were measured daily using a spectrophotometer (see paragraph 30 and Table 4).

24. Prior to the experiment, the relationship between absorbance and dry weight of suspended particulates had been determined by collecting triplicate samples of suspended sediment directly from the diluter or by preparing dilutions from the highest concentration. The dry weight of these samples was measured using the methods reported in Lake et al. (1985) and their absorbance was measured spectrophotometrically. Linear regression analysis of the data established the relationship between absorbance and dry weight. Analysis of variance and multiple comparison tests were performed on the suspended particulate data collected daily during the experiment.

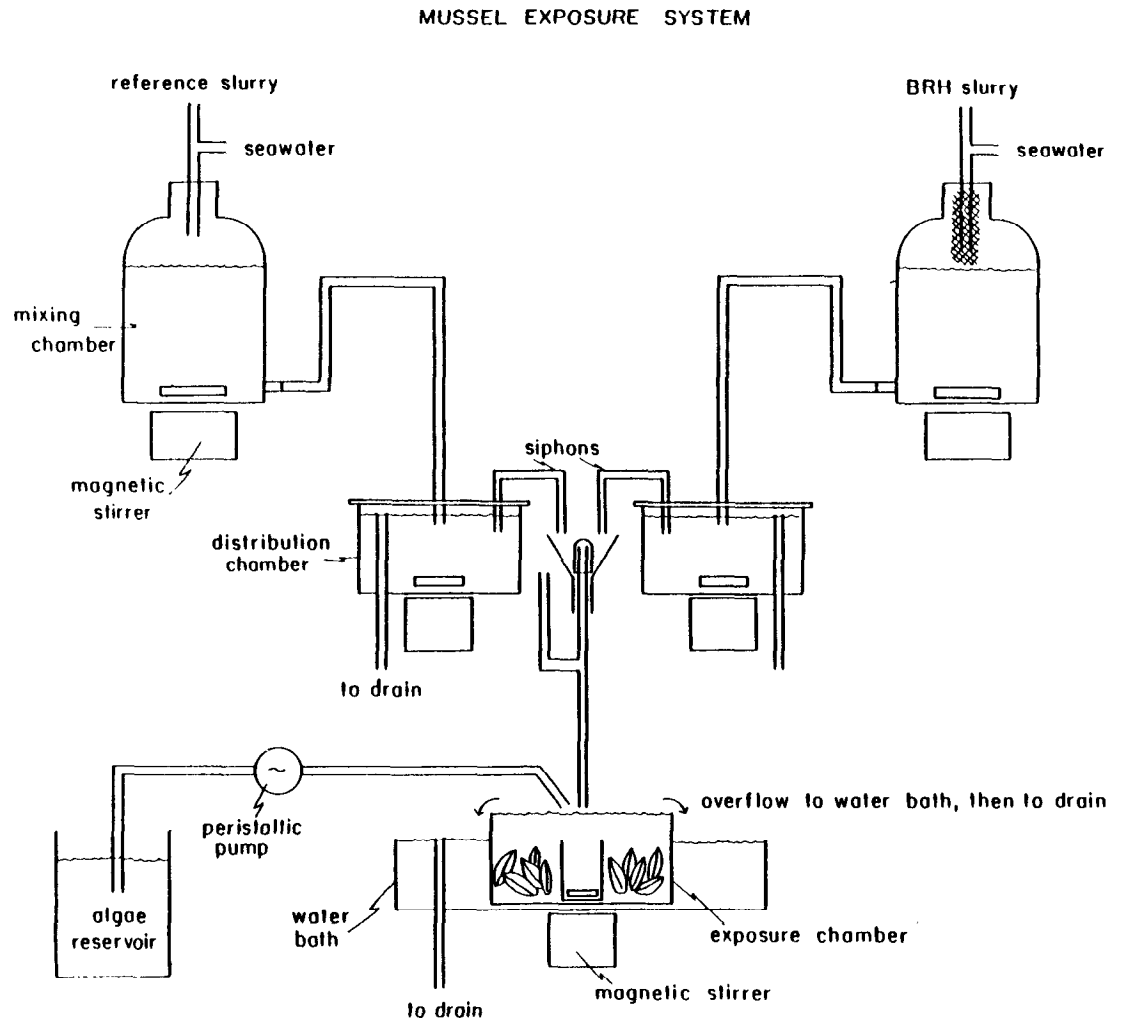

Figure 1. Sediment dosing system used for Mytilus edulis experiments.

25. For experiments A and B (see paragraph 21), the three exposure treatments consisted of (a) 100 percent BRH sediment (100 BRH), (b) 100 percent REF sediment (100 REF), and (c) a 50 percent-50 percent mixture of each sediment (50-50 BRH/REF). The total suspended solids concentration

was 50 mg/ℓ. Forty mussels were exposed in each treatment and fed Isochrysis aff. galbana (T-Iso) at a rate of 94 mg/mussel/day. On day 28, 15 mussels from each treatment were sampled for histopathological analysis.

Histological methods

26. Mussels were opened by forcing a sharp quahog knife between the two valves slightly above the byssus threads so that the tip of the knife is between the mantle and the shell. With a sweeping motion, the muscle and mantle were cut loose from the shell. Mussels were trimmed by cutting the body mass sagittally into 4 sections. The following conditions were noted while fixing and trimming mussels: (a) Malformations, especially in the gills and body mass, (b) Parasites, both external and internal, (c) Firmness of tissue, (d) Changes in the color of the internal organs such as the digestive diverticula and gonadal tissue, and (e) Cyst and tumor formation.

27. Fixation, dehydration (S-29), clearing (UC-670), paraffin infiltration, embedding, sectioning, and staining were done according to accepted methods (Yevich and Barszcz, 1981, 1983). Processing on the Autotechnicon tissue processor was according to the night run schedule on Table 3. An individual sample moves automatically and sequentially through the series of beakers. Because paraffin is not miscible with the S-29 dehydrating agent, a paraffin miscible clearing agent (UC-670) is necessary.

28. Based on our trimming techniques, four blocks were prepared for each mussel. Two sections from each block, cut at 6 microns, were put

on each slide. All blocks were saved and were recut as needed to provide tissue for the special staining techniques used to clarify histopathological findings (Yevich and Barszcz, 1981).

29. For this study, over 400 mussel slides were examined. These slides allowed examination of representative samples of all organs and tissues including the reproductive tract, the cardiovascular system (auricle, ventricle, and pericardial wall), the gills including cilia and mucous secretory cells, the kidneys, the gastrointestinal tract including the labial palps, the esophagus, the stomach, the intestine and digestive diverticula ducts and tubules, the byssus organ, the muscles, and the connective tissue.

Table 3

Routine Processing Schedules

Night Run			Day Run			Short Run*		
Beaker #	Reagent	Time	Beaker #	Reagent	Time	Beaker #	Reagent	Time
1	S-29	1 hr.	1	S-29	1/2 hr.	1	S-29	10 min.
2	S-29	2 hr.	2	S-29	1/2 hr.	2	S-29	10 min.
3	S-29	2 hr.	3	S-29	1/2 hr.	3	S-29	10 min.
4	S-29	2 hr.	4	S-29	1/2 hr.	4	S-29	10 min.
5	S-29	2 hr.	5	S-29	1/2 hr.	5	S-29	10 min.
6	S-29	2 hr.	6	S-29	1/2 hr.	6	S-29	10 min.
7	UC-670	1/2 hr.	7	UC-670	1/2 hr.	7	UC-670	10 min.
8	UC-670	1 1/2 hr.	8	UC-670	1 hr.	8	UC-670	10 min.
9	Paraffin	1 hr.	9	Paraffin	1 hr.	9	Paraffin	10 min.
10	Paraffin	1 hr.	10	Paraffin	1/2 hr.	10	Paraffin	10 min.
11	Paraffin	1/2 hr.	11	Paraffin	1/2 hr.	11	Paraffin	10 min.
Vacuum	Paraffin	1/2 hr.	Vacuum	Paraffin	1/2 hr.	Embed as quickly as possible		

* Delicate tissues.

Exposure system monitoring

30. Data from the daily monitoring of exposure conditions for BRH sediment experiments A and B are presented in Table 4.

Table 4

Daily monitoring data for Black Rock Harbor Experiments A and B*

	Treatment		
	100 REF	50-50 BRH/REF	100 BRH
Experiment A			
Actual incoming concentration (mg particulates/ℓ)	56.2 (8.2)	59.4 (5.5)	62.8 (9.9)
Actual surrounding concentration (mg particulates/ℓ)	11.6 (5.1)	24.5(15.4)	30.2(17.5)
Experiment B			
Actual incoming concentration (mg particulates/ℓ)	49.4 (6.1)	52.9 (5.7)	56.2 (8.6)
Actual surrounding concentration (mg particulates/ℓ)	14.1 (6.4)	23.5(10.1)	29.0(11.4)

* Values are means with standard deviation in parentheses.

Ampelisca abdita

Collection and holding

31. Sediment containing Ampelisca abdita was collected from Narrow River, Rhode Island, and transported unsieved to the laboratory. Collection dates and temperatures are shown for each test in Table 5. Salinity at the site typically ranged from 19.0-28.0 °/oo. The sediment was then sieved and the recovered animals were placed in presieved native or reference sediment for acclimation. These animals were acclimated to the test temperature (20°C) at the rate of 1°C/day in unfiltered seawater which ranged from 28 to 32 °/oo. During acclimation A. abdita were fed daily, ad libitum, with the diatom Phaeodactylum tricornutum.

Exposure methods

32. Two types of exposure chambers were used in these experiments. One was used for acute or short-term exposures and the other for chronic or long-term exposures greater than 28 days. Although no histological studies were conducted on chronically exposed amphipods, the chronic chambers were used in some short-term tests in conjunction with the acute chambers to insure that the chamber design did not adversely affect the test organisms. In the majority of experiments, the exposure system consisted of a 3.8 liter jar containing two exposure chambers (Figure 2). The exposure chambers consisted of 0.24 liter (8 oz.) glass jars, with four 2.5 cm diameter holes covered with 0.4 mm mesh nylon screening, fitted with polypropylene lids and self-starting siphons. The water (and particulates) flowed through the screens, into the exposure chambers, and out through the siphons to the drains

Table 5

Treatment Conditions and Percent Mortality for Solid and Suspended Phase Exposures of Black Rock Harbor Sediments to the Amphipod, Ampelisca abdita. Tests were run at 20°C. Test Duration was Four Days with the Exception of Test A which was 10 Days

Collection date/temp (°C)	Test	Date	Size (mm) $\bar{x} \pm SD$	Suspended Solids (mg/1)	Treatment (Suspended/Solid)	Number Exposed (N)
82/09/01 24°C	A Solid	82/12/03	5.3 ± 0.69	0.0	0%BRH/100%REF	80
				0.0	0%REF/12%BRH	80
82/09/01 24°C	B Solid	83/02/01	6.8 ± 0.77	0.0	0%BRH/100%REF	40
				0.0	0%REF/12.5%BRH	40
				0.0	0%REF/25%BRH	40
83/02/04 5°C	C Suspended	83/03/18	6.9 ± 0.92	22.4	0%BRH/100%REF	80
				22.7	33%BRH/100%REF	81
				18.9	66%BRH/100%REF	80
				18.2	100%BRH/100%REF	80
83/02/04 5°C	D Suspended	83/05/02	3.4 ± 0.34	16.0	0%BRH/100%REF	80
				12.9	100%BRH/100%REF	80
83/08/29 20°C	E Suspended	83/08/29	3.9 ± 0.53	165.3	0%BRH/100%REF	50
				157.9	25%BRH/100%REF	50
				148.3	50%BRH/100%REF	50
				149.5	75%BRH/100%REF	50
				75.3	100%BRH/100%REF	50
83/08/29 20°C	F Suspended	83/08/29	3.9 ± 0.53	214.6	0%BRH/100%REF	50
				211.1	25%BRH/100%REF	50
				218.8	50%BRH/100%REF	50
				223.8	75%BRH/100%REF	50
				138.0	100%BRH/100%REF	50

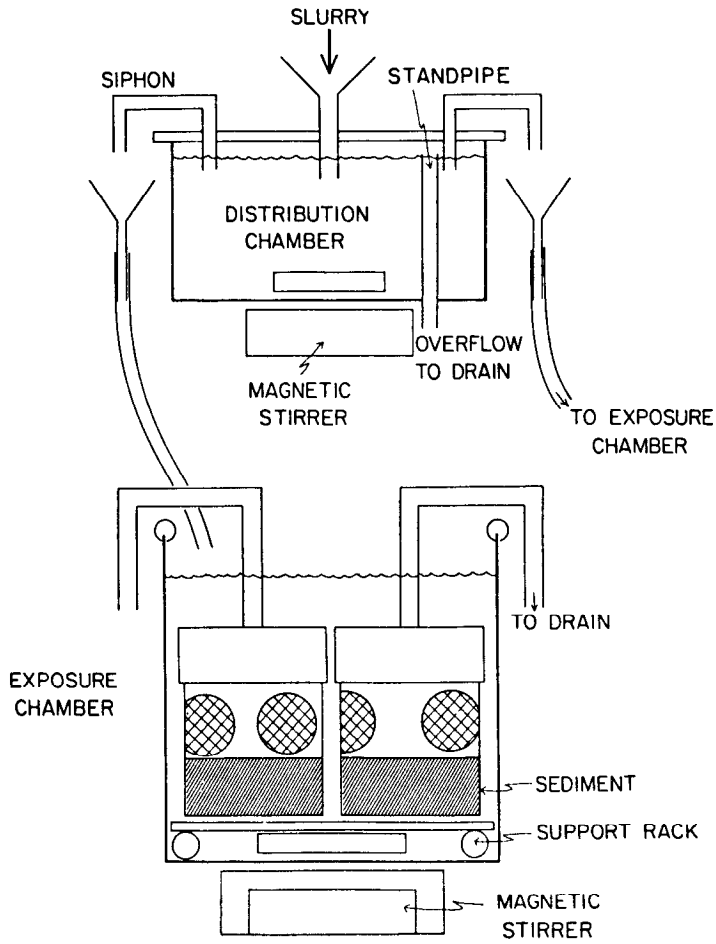

Figure 2. Distribution and exposure chambers used for solid phase and suspended particulate phase exposure of Y. limatula and A. abdita

(Figure 2). Experimental sediments were put in the bottom of the containers up to the lower edge of the screen openings. In one set of experiments, a different exposure system was used. This system was designed for long-term, chronic exposures of 28 days or longer and consisted of a 3.8 liter jar with 5 cm of sediment in the bottom. Overflow water (and particulates) were removed by siphon to a water trap which captured any entrained amphipods (Figure 3). Particulates were kept in suspension by vigorous aeration.

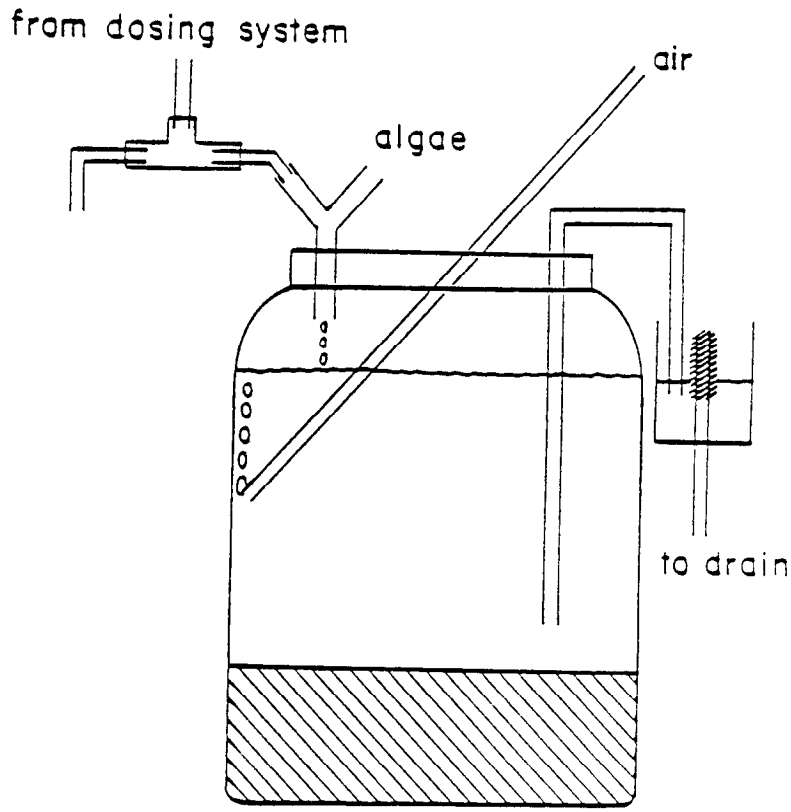

Figure 3. Chronic exposure chambers used for short-term tests with A. abdita

33. Experiments were conducted with two exposure regimes. First, amphipods were exposed to bedded BRH contaminated sediments (i.e., solid phase exposures) with filtered seawater flowing over them (Table 2, Tests A and B). In the second exposure regime, the solid phase was REF sediment but with a suspended particulate phase that consisted of proportions of BRH and REF sediments (Tests C-F).

34. The solid phase exposures were made up by mixing REF and BRH sediment in different volume proportions. These sediments were then placed in the bottom of the acute exposure containers and left in flowing seawater overnight to allow for the aeration of the surface sediments. As there were considerable mortalities at BRH concentrations of 50 percent

or above, only organisms exposed to lower concentrations were used for histological analysis. The treatment conditions for the two solid phase exposures reported here are shown in Table 5 (Tests A and B). Clean filtered seawater was delivered to the exposure systems at 40 ml/min.

35. The suspended phase tests had two general treatment levels: low particle densities and high particle densities. The first set of tests (C and D) were in the 20-mg/l range where a constant particle density was supplied to the distribution chambers (Figure 2) and then by siphon to the exposure systems. To achieve varying degrees of exposure to the dredged material while maintaining constant particle densities, a siphon and collection tube from both suspension systems (REF and BRH) were directed to a single exposure chamber. For example, to get one-third BRH and two-thirds REF (33 percent BRH exposure) at 60 ml/min, 20 ml/min of BRH and 40 ml/min of REF would be combined. A 66 percent exposure would be just the opposite: 40 ml/min BRH and 20 ml/min REF. Flow rates were measured daily and dry weights (mg/l) were measured biweekly using techniques described in Rogerson, Schimmel, and Hoffman (1985). Treatment conditions for the first series of suspended phase tests are shown in Table 5 (Tests C and D). The acute exposure chambers as shown in Figure 2 were used for these tests.

36. The second series of suspended phase tests (E and F) were run at considerably higher suspended solids concentrations. For these tests, the dosing system was similar to that described in Figure 1, except that the exposure system was that designed for chronic exposures (Figure 3). The particle densities and treatment conditions are shown

in Table 5 (Tests E and F). In all the suspended phase tests, the solid phase sediments were REF sediment.

37. At the beginning of each experiment, acclimated organisms were sieved from their holding sediments, sized, and the remainder sequentially distributed into 100-ml plastic beakers. Two or more beakers of experimental organisms were preserved for size determination. The remaining organisms were transferred to experimental chambers and checked after 1 hr in order to replace any organisms that had not burrowed. Two exposure chambers per treatment were placed in the exposure system.

38. Exposure chambers were checked daily and the number of individuals dead, on the sediment, on the water surface, and moribund were recorded. The number of molts and the condition of the tubes constructed were also monitored. At the conclusion of each assay, the sediment in all containers was sieved and the recovered animals were counted. Any animals missing or unaccounted for were assumed to be mortalities. The survivors were then preserved for histological analysis.

Histological methods

39. All surviving Ampelisca were removed from the exposure system and placed in vials of Dietrich's fixative. Because of differential survival between treatments, the number of animals varied per vial. After the animals were examined briefly for discolorations of the exoskeleton and gills, ulcerations, and malformations, a few drops of eosin were added to each vial, staining the animals to make them more visible during the embedding process.

40. Dietrich's fixative (Gray, 1954) was the fixative of choice for Ampelisca, as it does not harden the tissues as fast as Helly's, thus making it easier to control fixation (Barszcz and Yevich, 1976). It also helps to decalcify the chitin of the exoskeleton so that no other decalcifying reagent is needed.

41. The Ampelisca were left in Dietrich's fixative for several days during which time the exoskeleton was decalcified by the glacial acetic acid in the fixative. After fixation, all the amphipods from each exposure group were placed in a nylon netting envelope, washed in running water for 24 hours, stored in 70% ethanol, and then processed on the Autotechnicon tissue processor as described in the short run schedule of Table 3 (Barszcz and Yevich, 1976). All the animals from each exposure group were embedded in separate Paraplast blocks. Step-serial sections of tissues for three slides were cut from each block. These step-serial sections allowed observation of the organs at different levels in all the animals from each exposure treatment in a series of 3 slides. All slides were stained with Harris hematoxylin and eosin. The blocks were saved so that if more information was needed, the tissues could be recut and special stain performed to clarify and confirm the observations.

42. Sixty-three slides of Ampelisca were examined. The organ systems examined on each slide included the nervous system, eyes, gastrointestinal tract, mucous cells and glands, muscle, kidney, and reproductive tract.

Yoldia limatula

Collection and holding

43. Yoldia limatula were sieved from REF sediments onboard ship in January (5.7°C, 25 °/oo), February (1.1°C, 28.7 °/oo), and April (4.4°C, 26.8 °/oo) 1983. The organisms were then returned to the laboratory where they were sorted from shell material and placed in containers of REF sediment. The newly collected organisms were acclimated to 20°C at the rate of 1°C/day.

Exposure methods

44. Solid phase toxicity tests were conducted with Yoldia for 10 days at 20°C. The exposure system consisted of three 70- by 50-mm glass crystallizing dishes containing test sediment (65 mm deep) placed on a glass rack 3 cm off the bottom of a 3.8-ℓ glass jar with a siphon to drain. Filtered seawater entered the exposure system at a flow rate of 45 to 80 ml/min and was circulated within the system by a Teflon-coated stir bar operated by a water-driven stirrer. Each exposure system was placed in a 20°C water bath of recirculating seawater and monitored with a continuous temperature recorder. Organisms were added 6 to 24 hrs after the sediments were distributed to the exposure chambers. Experimental concentrations were 100, 66, 50, 33, 25, and 0 percent BRH mixed with a complementary amount of REF sediment. The water content was determined for all sediment combinations. Each treatment consisted of two replicates. A minimum of two replications of each test was conducted to satisfy statistical design criteria.

45. Suspended particulate toxicity tests with Yoldia were conducted for 10 days at 20°C. For the suspended particulate exposures, 25 mg/l of 100 percent BRH and 100 percent REF slurries were each delivered to a distribution chamber (17 cm x 9 cm) fitted with a standpipe to maintain a constant water level as shown in Figure 2. Material was kept in suspension with a water-driven stirrer and stir bar. To adjust flow rates to each exposure container, U-shaped glass siphons were set at the desired height. The suspension was collected by small glass funnels that drained through polypropylene tubing to the exposure chambers. Flow rates were measured daily. Sediment suspensions were monitored at least two times during each test using dry weight measurements. At the end of each test, the Yoldia were sieved from the test sediment, mortalities were recorded, and the survivors were preserved for histological analysis.

Histological methods

46. Yoldia (up to 1.5 cm) were dropped into fixative after their shells had been cracked slightly, but not crushed. This allowed the fixative to penetrate the tissue as the animals were easier to remove from the shell after fixation. They were sectioned sagittally, or if very small, processed whole. If the Yoldia were too small to open and remove from their shells, after the appropriate fixation time (6-12 hours), they were removed from the fixative and placed in decalcifying fluid. Decalcification was completed when no hard areas of shell remained. After decalcification, the animals were stained with eosin, put in net bags and then into cassettes, washed for 24 hours, and either

stored in 70% ethanol or processed on the Autotechnicon according to the day run schedule on Table 3. All the animals in each exposure group were embedded in one block. Enough step-serial sections were cut from each block to make three slides. The slides were stained with Harris hematoxylin and eosin.

47. Eighty-four slides from Yoldia were examined. The organs and tissues observed included the reproductive tract, cardiovascular system, gills, kidney, gastrointestinal tract, muscle, and connective tissue.

Nephtys incisa and Neanthes arenaceodentata

Collection, culture, and holding

48. Two species of polychaetes were used, Nephtys incisa and Neanthes arenaceodentata Moore (Nereis acuminata Ehlers, Nereis caudata delle Chiaje). Nephtys incisa, indigenous to the disposal area in Central Long Island Sound, were collected with a Smith-McIntyre grab sampler (0.1 m^2) from the South reference site on August 2 and September 15, 1983. Worms were sieved (0.55 mm mesh sieve) from the sediment on board ship, sorted into general size classes, placed into sediment from the collection station, and transported back to the laboratory. Worms were held in the sediment with filtered Narragansett Bay water at 20°C flowing over the sediment surface. No temperature acclimation was needed because the collection and test temperatures were the same. Nephtys incisa were fed prawn flakes (ADP - Prime, Aquatic Diet Technology, Inc., Brooklyn, NY) directly into the sediment surface during holding. At the start of the test, worms were sieved

(0.35 mm mesh sieve) out of the sediment and placed in the test chambers. Neanthes arenaceodentata is a species kept in laboratory culture at the same salinity and temperature used in these tests. Details of culture methods and conditions have been published by Reish (1980). Nutritional requirements were determined by Schauer and Pesch (personal communication). For both species, all tests were conducted with juvenile worms.

Exposure methods

49. Two 10-day suspended particulate tests were performed with each species at 20°C. The two tests with N. incisa were conducted on worms collected at the two field sampling times (Table 6).

50. The suspended sediment experimental system consisted of three modules: the controlled dosing system, the dilution and distribution system, and the test chambers. Two identical dosing systems, one for REF and one for BRH, provided a constantly recirculating source of concentrated sediment slurry (in seawater) passing by a three-way valve, which led to the dilution and distribution system (Rogerson et al., 1985). Argon gas was added to the reservoir of the dosing system to minimize oxidation of the slurry. The three-way valve was controlled by a microprocessor programmed to deliver a pulse of slurry at periodic intervals. In the dilution and distribution system, the concentrated slurry was mixed with seawater to the proper concentration of suspended solids and distributed to the individual test chambers. Actual concentration of suspended particulates in the test chambers was determined (by dry weights) periodically.

51. The test chambers were glass crystallizing dishes (150 by 75 mm), which contained 400 ml of sediment (2.5 to 3.5 cm deep). Each dish contained a smaller glass crystallizing dish (60 by 35 mm) in the center of the larger dish. A Teflon-coated stir bar was placed in the small dish, which received the inflow water, to keep the particulate material in suspension. The inflow water flowed out of the central dish over the sediment surface, and overflowed the edge of the large crystallizing dish.

52. Exposure conditions for the solid phase portion of the suspended particulate tests were 100 percent REF or 100 percent BRH sediment. These two solid phase exposure conditions in combination with the two suspended sediment exposures, REF or BRH at a nominal concentration of 200 mg/l (dry weight), gave a total of four treatments. The measured concentrations are given in Table 6. The exposure conditions for these experiments were chosen on the basis of previous experiments and were expected to be sublethal for the 10-day exposure period. Survival of N. incisa was 100 percent in all treatments except the BRH/REF treatment in the first experiment where it was 97 percent. In the experiments with N. arenaceodentata, survival was 87 percent or better in all treatments.

53. The worms were fed prawn flakes (ADT-Prime, Aquatic Diet Technology, Brooklyn, NY) in a suspension of seawater, which was pumped by peristaltic pump into the distribution chamber of the dosing system. The amount fed was 127 mg (dry weight) per test chamber per day. This amount of food was determined based on prior feeding studies with N. incisa (personal communication, P. Schauer).

Table 6

Measured Concentrations (Dry Weight) of Suspended Particles for Experiments with Nephtys incisa and Neanthes arenaceodentata

Date of Test	Species	Treatment (suspended/solid)	Concentrations (mg/l) $\bar{x}$	SD	Number Animals[a]
83/09/02	N. incisa	REF/REF	211	87	15
		BRH/REF	171	53	14
		REF/BRH	211	87	15
		BRH/BRH	171	53	15
83/09/20	N. incisa	REF/REF	199	73	10
		BRH/REF	226	47	10
		REF/BRH	199	73	10
		BRH/BRH	226	47	10
83/08/16	N. arenaceo-dentata	REF/REF	217	86	15
		BRH/REF	190	61	13
		REF/BRH	217	86	15
		BRH/BRH	190	61	12
83/09/22	N. arenaceo-dentata	REF/REF	199	73	15
		BRH/REF	222	44	15
		REF/BRH	199	73	15
		BRH/BRH	222	44	11

[a] Number of animals examined by histopathological techniques out of a total of 30 worms, the remainder were used for other purposes.

54. During the tests, all dishes were examined daily for the appearance of any worms on the surface of the sediment, but none were seen. Then the sediment was sieved (0.35 mm mesh) and the worms retrieved, counted, and preserved. Worms missing were presumed dead.

55. All tests were conducted with sand-filtered Narragansett Bay seawater at 20°C and approximately 30 °/oo salinity. Flow rates were about 35 ml/min. The photoperiod was a 14:10 hr light-dark cycle.

Histological methods

56. Individual Nephtys and Neanthes were fixed in Helly's fixative. Worms less than 3 cm long were fixed whole by placing them directly into fixative. They were processed and embedded without trimming. Larger worms, after initial fixation, were cut sagittally into sections about 3 cm long. When trimming, sagittal sections were taken from each animal. While fixing and trimming the worms, observations were made for the following conditions: (a) Discoloration of the epidermis, (b) Ulcerations of the epidermis, (c) Malformation of the parapodia, and (d) Parasites both external and internal.

57. Fixation time varied from one hour for small worms to 14 hours for the larger, thicker sections. After fixation, they were washed, stored in 70% ethanol, and processed on the Autotechnicon on the overnight run (Table 3). After embedding, tissues for three slides were cut from each block, which allowed all organ systems to be examined. All slides were stained with Harris hematoxylin and eosin.

58. Over 650 slides were examined of the two species of worms. Among the organs and tissues examined were the epidermis, muscle, gastrointestinal tract, nervous system, reproductive tract, and mucous secretory cells.

PART III: RESULTS AND DISCUSSION

Mytilus edulis

59. The histopathologic results for Mytilus edulis exposed for 28 days to Black Rock Harbor dredged material are presented in Table 7. In Experiment A, histological changes were noted in the reproductive tract of one of the four females (25%) exposed to 50% REF/50% BRH. In the affected animal, the ova were basophilic, showed a loss of nuclei, lacked normal cytoplasm and vitelline membrane (Figure 4). This condition became more severe in the 0% REF/100% BRH treatment where seven of the eight females (87%) exposed had changes in their reproductive tracts. Of these females, one (12%) had basophilic ova in dilated follicles similar to those described above. Six of the eight females (75%) exposed had varying number of vacuolated ova (Figure 5). The second organ system showing histopathological changes in Experiment A was the heart. Two animals (13%) exposed to 0% REF/100% BRH (Table 7) had swelling of some of the muscle bundles in the auricle. This swelling was characterized by the presence of ground substance with stellate and spindle shaped cells containing fibers (Figure 6). No changes were detected in either the 50% REF/50% BRH or 100% REF/0% BRH treatments.

60. Examination of the results from Experiment B indicate that the only histopathological changes were associated with the heart. These changes were tumors of a mixed variety of cauliflower-like and pedunculated growths arising from the auricle, ventricle, or pericardial walls (Figure 7). The tumors consisted of an amorphous pink staining ground substance containing fibers and stellate and spindle shaped cells which appeared to be fibroblasts. Red granular amebocytes

Table 7

Histopathological findings in Mytilus edulis exposed for 28 days to Black Rock Harbor Dredged Material

Organs Examined	100% REF/0% BRH		50% REF/50% BRH		0% REF/100% BRH	
Experiment A						
Reproductive tract						
Males	N*= 8	0%	N = 11	0%	N = 7	0%
Females	N = 7	0%	N = 4	25%	N = 8	87%
Heart		0%		0%		13%
Gills		0%		0%		0%
Kidney		0%		0%		0%
Gastrointestinal Tract		0%		0%		0%
Muscle		0%		0%		0%
Experiment B						
Reproductive tract						
Males	N = 10	0%	N = 9	0%	N = 9	0%
Females	N = 8	0%	N = 8	0%	N = 8	0%
Heart		0%		0%		23%
Gills		0%		0%		0%
Kidney		0%		0%		0%
Gastrointestinal Tract		0%		0%		0%
Muscle		0%		0%		0%
Byssus Organ		0%		0%		0%

* N = Number of animals examined.

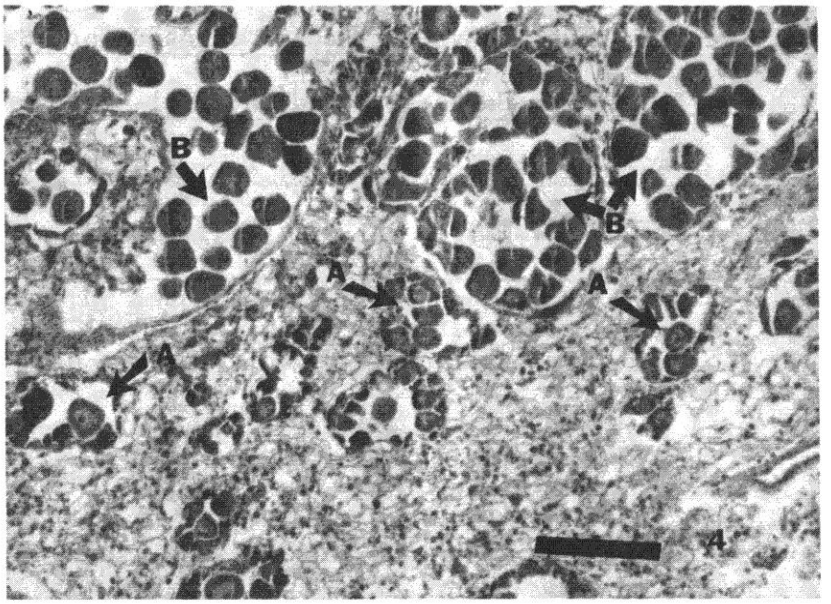

Figure 4. Portion of the reproductive tract of a female Mytilus edulis exposed to 50%REF/50%BRH. Arrow A indicates normal ova. Arrows B indicate large basophilic ova lacking nuclei, normal cytoplasm, and vitelline membrane. Hematoxylin and eosin stain. Scale bar = 200 μm.

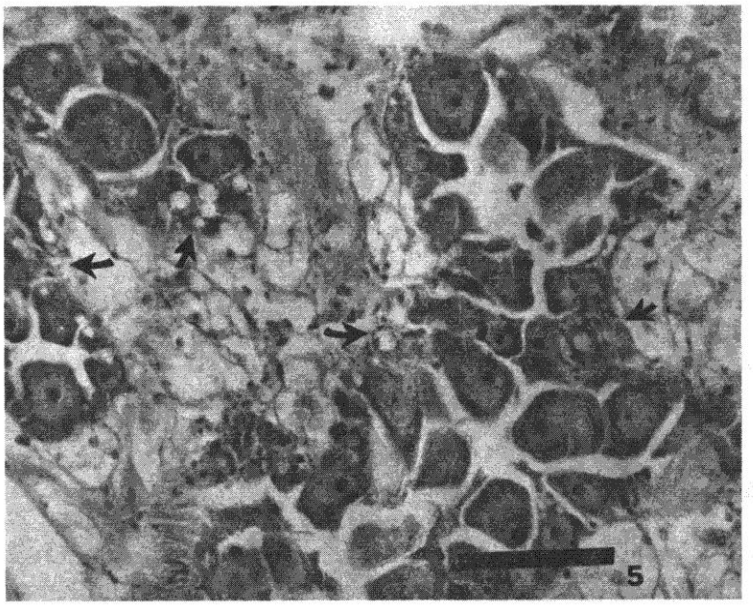

Figure 5. Portion of the reproductive tract of a female Mytilus edulis exposed to 0%REF/100%BRH. Arrows indicate the vacuolated areas in the small ova lining the follicle walls. Hematoxylin and eosin stain. Scale bar = 100 μm.

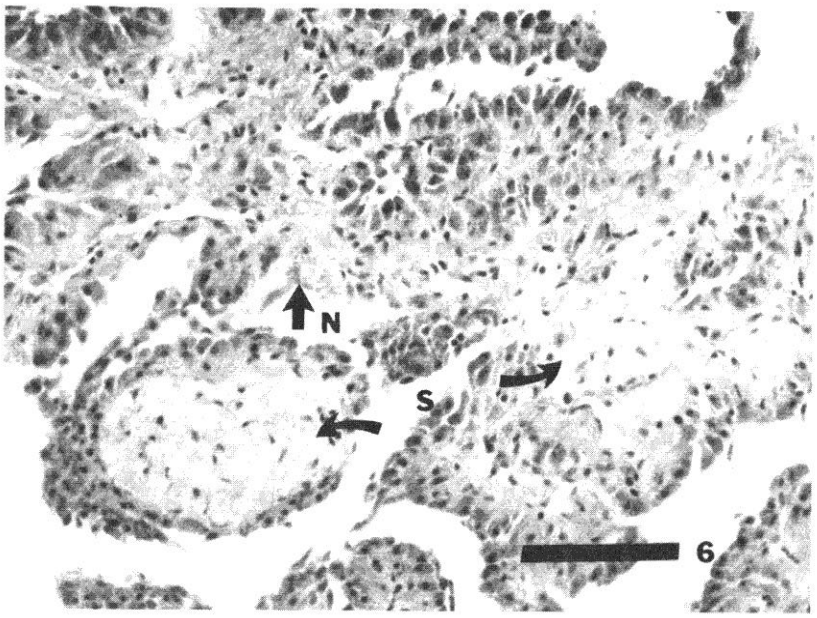

Figure 6. Portion of auricle from a Mytilus edulis exposed to 0%REF/100%BRH, Experiment A. Arrow S indicates swelling of the heart muscle bundles. Arrow N indicates normal muscle bundles. Hematoxylin and eosin stain. Scale bar = 100 μm.

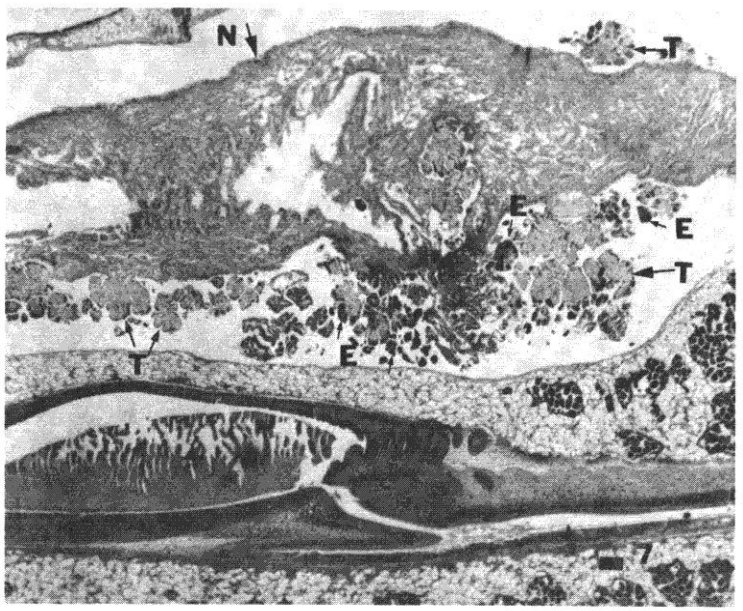

Figure 7. Section of the pericardial cavity of a female M. edulis exposed to 0%REF/100% BRH, Experiment B. Note cauliflower-like tumor formations (T) from various portions of the ventricle and auricle, arrow E indicates proliferation of epithelial cells. Hematoxylin and eosin stain. Scale bar = 200 μm.

were also seen individually or in groups in the ground substance. Proliferation of the epithelial cells, also known as red gland or pericardial gland cells, was noted surrounding the tumors (Figure 8). In one animal, along with the above described growths, there were several cauliflower-like growths which were a proliferation of the ventricle muscle bundles surrounded by a proliferation of the pericardial gland cell epithelium.

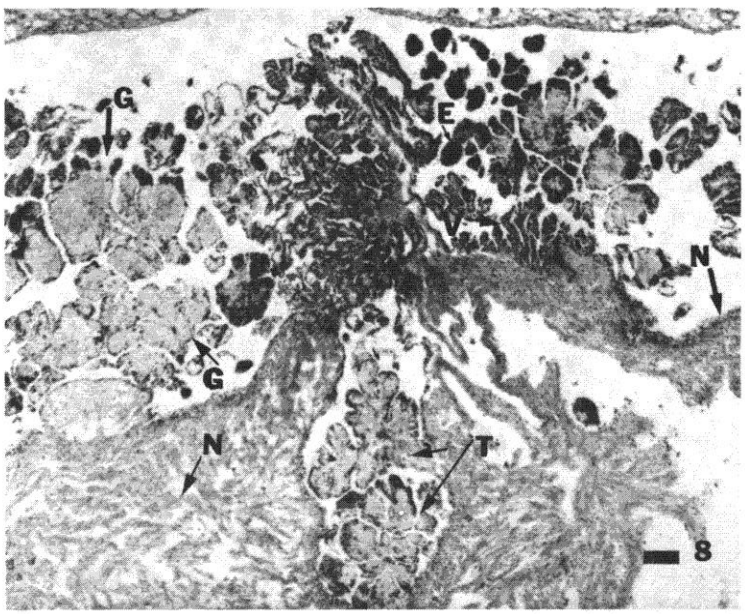

Figure 8. Section of the auricle and ventricle of M. edulis exposed to 0%REF/100%BRH, Experiment B. Arrows G indicate ground substance tumor; arrows E, the proliferation of epithelial cells; arrow V, proliferation of ventricle muscle bundles; and arrow N, normal areas. Hematoxylin and eosin stain. Scale bar = 100 µm.

61. The histological results from the exposure of M. edulis to BRH-suspended sediments can be viewed from two perspectives, (a) sensitivity of histological changes, that is, the ability of this technique to detect effects; and the reproducibility of the types of histological changes observed between experiments. Examining the issue of sensitivity one can make the following observations. In Experiment A, the observed reproductive responses were dose related with a threshold value of 50% REF/50% BRH. This correlates well with observed changes in scope for growth where significant differences were reported between the 100% REF/0% BRH (Control) and both the 50% REF/50% BRH and 0% REF/ 100% BRH treatments. Experiment B showed histological changes (heart) only at the 0% REF/100% BRH treatment which corresponds with the results for scope for growth (Nelson et al., 1985). Therefore, when histological changes were detected in chronic exposures of M. edulis, they occurred at exposure concentrations similar to scope for growth.

62. The reproducibility of the histological results in the M. edulis experiments while not readily amenable to statistical comparison can be considered acceptable. Eight distinct organ/tissue systems were examined at three treatment combinations for two experiments. If normal tissue values are considered as equally important to histopathological changes, then only two of 48 possible organ-exposure-experiment combinations do not coincide. The combination of spawning condition and stress from BRH exposure in Experiment A, but not in Experiment B, may be responsible for the inconsistencies in noted reproductive changes. Normally, M. edulis spawns from September-November and spent gonadal tissue of the female is resorbed. The resorption process and stress

conditions mimicing resorption can produce histological changes similar to those reported in Experiment A. Bayne et al. (1978) has demonstrated that a variety of types of stress have a significant impact on the regression and resorption of previously formed gametes. However, there is consistent agreement between all other treatments and a high degree of similarity in the changes reported for the heart in both experiments.

63. The degree of reproducibility is gratifying considering the subjective nature of the endpoint and the sample sizes examined. The changes reported in the heart and cardiovascular system were both qualitatively and quantitatively similar between Experiments A and B. Of equal importance is that normal histology was reported for the gills, kidney, gastrointestinal tract, and muscle. The reproducibility was excellent given the number of organ systems and treatments. The histological changes in the reproductive tract that were reported only for Experiment A and not B may result from the combination spawning condition and the stress of BRH exposure.

Ampelisca abdita

Solid phase

64. Histopathological changes occurred in the gills of adult amphipods (length = 5.3 mm) exposed for ten days to bedded sediments containing 12% by volume BRH-dredged material. All exposed organisms (N = 44) showed necrosis of the gill epithelium and disruption of the gill capillary network (Table 8). Organisms exposed to 100% REF control showed normal gill histology. In addition, 10% of the organisms exposed

Table 8

Histopathologic Findings in Adult Ampelisca abdita Exposed to Black Rock Harbor Dredged Material

Organs Examined	Treatments (Suspended/Solids)		
	Experiment A, Solid (10 day exposure)		
	0% BRH/100% REF N*=77	0% REF/12% BRH N=44	
Nervous system	0%	0%	
Eyes	0%	0%	
Gastrointestinal tract	0%	0%	
Mucous cells and glands	0%	10%	
Muscle	0%	10%	
Kidney	0%	0%	
Reproductive tract	0%	0%	
Gills	0%	100%	
	Experiment B, Solid (4 Day Exposure)		
	0% BRH/100% REF N=25	0% REF/12.5% BRH N=14	0% REF/25% BRH N=5
Nervous system	0%	0%	0%
Eyes	0%	0%	0%
Gastrointestinal tract	0%	0%	0%
Mucous cells and glands	0%	0%	0%
Muscle	0%	0%	0%
Kidney	0%	0%	0%
Reproductive tract	0%	0%	0%
Gills	0%	0%	0%

* N = Number of animals examined.

to the 12% BRH treatment showed alterations in the muscle bundles and atrophy of the mucous tube glands.

65. When the animals were exposed to 0% BRH, 12.5% BRH and 25% BRH for a period of 4 days (Experiment B), histopathologic examination showed no differences between the 0% BRH exposed animals and those exposed to 12.5% BRH and 25% BRH. These animals were of similar size and life stage to those used in Test A. Test B amphipods were acclimated in the laboratory for 2 months longer; the longer exposure period (10 days) of Test A is postulated to explain the difference in histopathological changes between these two tests.

Suspended phase

66. Adult amphipods (length 6.9 mm) exposed for 4 days to a range of suspended BRH concentrations at a total suspended solids concentration of 20 mg/ℓ showed no histopathologic differences between any of the exposed treatments and the control animals (Table 9, Experiment C). In contrast, when this experiment was repeated with growing juveniles (3.9 mm length), histopathological effects were seen in the 100%BRH/100%REF treatment, but not in the 0%BRH/100%REF control (Table 9, Experiment D). All animals (n = 73) pooled from both replicates of the 100%BRH/100%REF exposure (Experiment D) showed necrosis of the lamellae of the gills as well as loss of normal gill architecture (Figures 9 and 10). The digestive ceca in two animals showed necrosis and sloughing of the mucosa. Similarly, all animals exposed to BRH sediment had atrophied and vacuolated mucous cells and mucous tube glands, and there was a loss of the basophilic staining granular material.

Table 9

Histopathologic Findings in Ampelisca abdita Exposed to Suspensions of Black Rock Harbor Dredged Material

	Experiment C			
Adult	0%BRH/100%REF N*=65	33%BRH/100%REF N=68	66%BRH/100%REF N=70	100%BRH/100%REF N=60
Nervous system	0%	0%	0%	0%
Eyes	0%	0%	0%	0%
Gastrointestinal tract	0%	0%	0%	0%
Mucous cells and glands	0%	0%	0%	0%
Muscle	0%	0%	0%	0%
Kidney	0%	0%	0%	0%
Reproductive tract	0%	0%	0%	0%
Gills	0%	0%	0%	0%

	Experiment D	
Juvenile	0% BRH/100% REF N=77	100% BRH/100% REF N=73
Nervous system	0%	0%
Eyes	0%	0%
Gastrointestinal tract	0%	0%
Mucous cells and glands	0%	100%
Muscle	0%	0%
Kidney	0%	0%
Reproductive tract	0%	0%
Gills	0%	100%

*N = Number of animals examined.

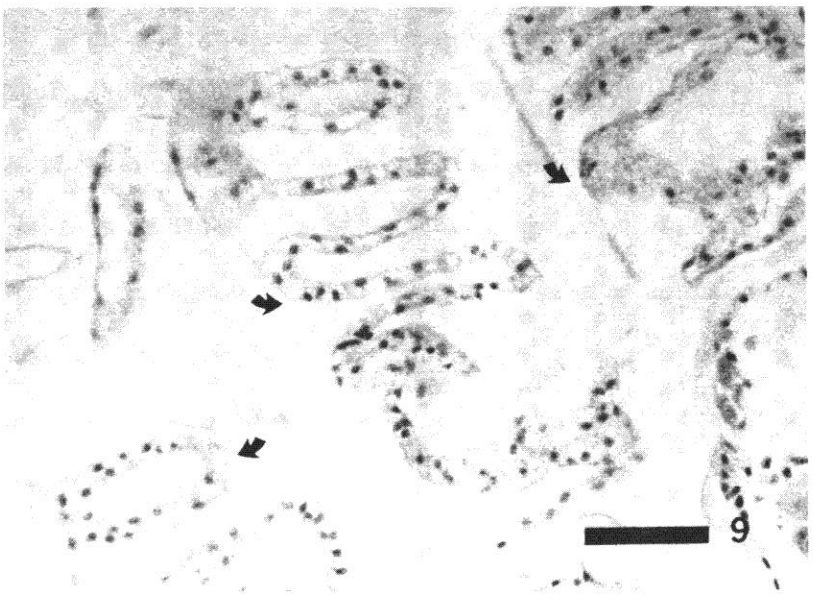

Figure 9. Gills of Ampelisca abdita exposed to suspended reference sediment. Arrows point to normal gills. Hematoxylin and eosin stain. Scale bar = 200 μm.

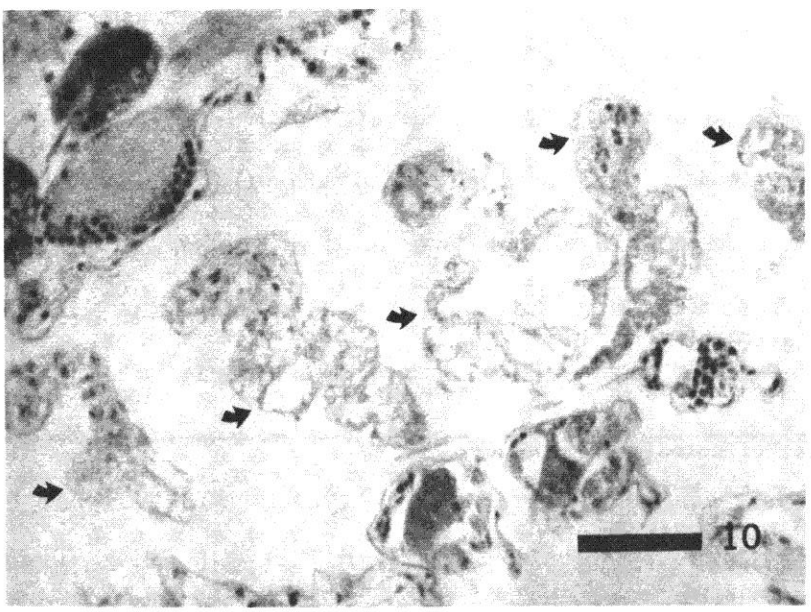

Figure 10. Gills of Ampelisca abdita exposed to suspended 100% BRH. Arrows point to necrotic gills and dilation of the blood vessels. Hematoxylin and eosin stain. Scale bar = 200 μm.

67. The differences in histopathological responses noted between the 100%BRH/100%REF treatment tests C and D appear to be correlated with differences in the laboratory acclimation period and the life stage of the amphipods used for each experiment. The Ampelisca for both tests were field collected in February and acclimated to 20°C. The organisms that were used in Experiment C, and showed no effects, were the field parental population that had grown to adult size (6.9 ± 0.92 mm). By the time the test was repeated (Experiment D), the parental field population had mated in the laboratory and produced young. It was the first filial generation of actively growing young (3.4 ± 0.34 mm) that were used in Experiment D. Although BRH sediments had no substantial effect on survival patterns in Experiments C and D, the more actively growing population in Experiment D showed an increased incidence of pathology. It is important to note that the types of pathologies, when occurring, are similar whether the exposure was from bedded sediment alone or by the suspended solids. Thus, qualitatively, there is a reasonable degree of similarity in responses between the two exposure routes.

68. Two definitive 96 hr acute toxicity studies (Table 10, Experiments E and F) were conducted to determine the reproducibility of the test method. Histological changes involving the necrosis of gill lamellae and loss of normal architecture were detected in all organisms exposed to 100% BRH/100% REF in both experiments. The mucous cells and tube secretive glands showed atrophy and vacuolization in all organisms exposed to 100% BRH/100% REF in both Experiments E and F. Two organisms (n = 44) from Experiment E showed necrosis of the ceca epithelium. These results indicate a high degree of concurrence

Table 10

Histopathologic Findings in Definitive Acute Toxicity Studies with Ampelisca abdita

Exposed to Black Rock Harbor Dredged Material

	Experiment E, Suspended				
	0%BRH/100%REF N*=49	25%BRH/100%REF N=49	50%BRH/100%REF N=41	75%BRH/100%REF N=43	100%BRH/100%REF N=44
Nervous system	0%	0%	0%	0%	0%
Eyes	0%	0%	0%	0%	0%
Gastrointestinal tract	0%	0%	0%	0%	4%
Mucous cells and glands	0%	0%	0%	0%	100%
Muscle	0%	0%	0%	0%	0%
Kidney	0%	0%	0%	0%	0%
Reproductive tract	0%	0%	0%	0%	0%
Gills	0%	0%	0%	0%	100%
	Experiment F, Suspended				
	0%BRH/100%REF N=42	25%BRH/100%REF N=43	50%BRH/100%REF N=31	75%BRH/100%REF N=28	100%BRH/100%REF N=30
Nervous system	0%	0%	0%	0%	0%
Eyes	0%	0%	0%	0%	0%
Gastrointestinal tract	0%	0%	0%	0%	0%
Mucous cells and glands	0%	0%	0%	0%	100%
Muscle	0%	0%	0%	0%	0%
Kidney	0%	0%	0%	0%	0%
Reproductive tract	0%	0%	0%	0%	0%
Gills	0%	0%	0%	0%	100%

* N = Number of animals examined.

in terms of the types and incidence of histological changes resulting from exposure to BRH sediments. The sensitivity, that is the concentration of BRH sediments eliciting a response, is analogous to that previously reported for acute mortality (Gentile et al., 1985). In fact, the histological results provide a possible explanation for the acute mortalities reported.

69. Generally, there is a well defined pattern of histological changes in A. abdita exposed to BRH sediments. For experiments and exposure concentrations in which pathological changes were elicited the following general observations can be made. The types of pathologies (gill, mucous tube cells) were similar whether bedded sediments or suspended sediments were the source of contamination. For bedded sediments, ten days of exposure were required to elicit a response in mature amphipods. In suspended phase experiments (C and D), the rapidly growing juveniles were more sensitive than the adults. When duration of exposure, size of organism, and exposure regimes were held constant (E and F), there was concurrence between experiments in terms of the types of pathologies. Histology provided a potential explanation for the observed mortalities. However, the presence of pathologies was not observed to be more sensitive than mortality in these short-term tests. Therefore, while providing insight into the causes of mortality, it did not provide an added measure of sensitivity. The degeneration of the mucous-tube-building glands could interfere with the organism's ability to build tubes. The inability to build tubes would leave the animals more exposed to the potentially toxic substances found in the BRH sediments and ecologically would make them more vulnerable to predation.

The necrosis of the gill lamellae and ceca would eventually lead to death. The breakdown of the tube building process is postulated as the primary factor in the greater acute lethality of BRH sediments to Ampelisca in the solid phase.

Yoldia limatula

70. Yoldia limatula were exposed for 10 days to bedded reference and Black Rock sediments. The exposures consisted of 100% REF and 100% BRH sediment and proportional mixtures by volume of these two sediments to produce 33% and 66% BRH concentrations. Forty individuals were exposed per treatment distributed in two replicates of twenty animals each. Survival was excellent with significant mortalities (35%) occurring only in the 100% BRH sediment treatment. Histological examinations were conducted on the gills, gastrointestinal tract, muscle, kidney, and reproductive tract. The results (Table 11) indicate no histopathologies detected in any organ system for any of the treatments. The limited availability of organisms precluded repeating this test in its entirety. However, in subsequent studies with Yoldia no histological effects were detected for 50% BRH bedded sediment exposures of 10 days duration. The lack of both histological effects and mortalities is surprising since this species is an infaunal deposit feeding bivalve mollusc that should have experienced maximal exposure under the experimental conditions. The obvious explanation was that they did not actively feed and process sediment in the high BRH exposures and thus did not receive the expected exposure. This is supported by

Table 11

Histopathological Findings in Yoldia limatula exposed for 10 days to solid phase Black Rock Harbor Sediment

Organs examined	Percent Abnormality			
	100% REF N*=40	33% BRH N=39	66% BRH N=37	100% BRH N=26
Gills	0%	0%	0%	0%
Gastrointestinal Tract	0%	0%	0%	0%
Muscle	0%	0%	0%	0%
Kidney	0%	0%	0%	0%
Reproductive Tract	0%	0%	0%	0%

* N = Number of animals examined.

Table 12

Histopathological Findings in Yoldia limatula Exposed for 10 Days to Suspended and Solid Phase Black Rock Harbor Sediment

Organs examined	Percent Abnormality			
	Susp.-REF / Solid-REF	Susp.-REF / 50%-BRH	Susp.-BRH / Solid-REF	Susp.-BRH / Solid-BRH
Gills	0%	0%	0%	0%
Gastrointestinal Tract	0%	0%	0%	0%
Muscle	0%	0%	0%	0%
Kidney	0%	0%	0%	0%
Reproductive Tract	0%	0%	0%	0%

results of feeding studies (Rogerson et al., 1985) and coupled with the short duration of the test probably accounts for the lack of results.

71. A second experiment was conducted to examine the impact of contaminated suspended sediments on the survival and histology of Yoldia limatula (Table 12). Suspended reference and BRH sediments at 25 mg/ℓ were used in conjunction with reference + 50% BRH bedded sediments. Survival was good in all treatment combinations except when BRH-suspended sediments were used with 50% BRH bedded, where mortalities reached 25%. As in the previous experiment, histological examinations were conducted on the gills, gastrointestinal tract, muscle, kidney, and reproductive tract. The results (Table 12) indicate no pathologies were detected in any organ system for any of the treatments. This was not unexpected in view of the results from the bedded sediment studies. Other studies with Yoldia conducted at 500 and 1000 mg/ℓ of BRH suspended sediments for 10 days also failed to produce either mortality or histological changes. In these studies there was no evidence of feeding and processing of sediment; consequently, there was limited exposure to the contaminants.

Nephtys incisa

72. Nephtys incisa were exposed to suspensions (≈ 200 mg/ℓ) of REF or BRH sediments in combination with either REF or BRH sediment solid phase for 10 days. In Experiment A (Table 13), no histopathological changes were noted between the worms from the different treatments. However, in Experiment B (Table 11), differences were seen in the worms exposed to BRH(suspended)/ BRH(solid phase). Five out of 10 (50%) of the animals in this treatment showed cellular debris and muscle degeneration in at

Table 13

Histopathologic Findings in Nephtys incisa Exposed for 10 Days to Black Rock Harbor Sediment

Organs Examined	Percent Abnormality			
	Experiment A			
	REF/REF N*=15	BRH/REF N=14	REF/BRH N=15	BRH/BRH N=15
Epidermis	0%	0%	0%	0%
Muscle	0%	0%	0%	0%
Intestine	0%	0%	0%	0%
Nervous system	0%	0%	0%	0%
Reproductive Tract	0%	0%	0%	0%
	Experiment B			
	REF/REF N=10	BRH/REF N=10	REF/BRH N=10	BRH/BRH N=10
Epidermis	0%	0%	0%	50%
Muscle	0%	0%	0%	50%
Intestine	0%	0%	0%	0%
Nervous system	0%	0%	0%	0%
Reproductive tract	0%	0%	0%	0%

* N = Number of animals examined.

least one of the parapodia. Muscle degeneration in the parapodia could interfere with the worm's ability to move and burrow which is essential to its survival.

73. If the histopathologic changes seen in N. incisa from the BRH/BRH treatment are due to contact with solid phase BRH sediment, then one would expect there to be similar changes in the worms exposed to the other treatments with BRH solid phase sediment. During the 10-day experiment, some of the suspended particulate material settled out and accumulated on the surface of the bedded (solid phase) sediment. By the end of the 10 days, about 1 cm of particulate matter had accumulated in all treatments. Observations of the burrows visible along the sides of the exposure chambers showed that worms in the REF/BRH treatment had moved up into the accumulated 1 cm layer of REF sediment, and thus apparently minimized their contact with BRH bedded sediment. Also, most of the burrows visible along the sides of the dishes in the BRH/REF treatment were below the surficial BRH sediment in the REF sediment. Only a few single burrows that extended straight up through the accumulated BRH layer were visible. Therefore, again it appeared that these worms had minimized their contact with the BRH sediment and thus effectively reduced their exposure.

74. We cannot explain why we saw differences in worms from the BRH/BRH treatment in Experiment B and not in Experiment A. The worms for each experiment were collected at different times but only 3 weeks apart. The acclimation times in the laboratory were similar, 7 and 5 days for Experiments A and B, respectively, and no mortalities were detected in either experiment. Also, the worms used were similar in

size, mean wet weight of 0.082 and 0.087 gm for the BRH/BKH treated worms for Experiment A and B, respectively. All of the BRH sediment for the solid phase portion of both experiments came from the same barrel, but were from different jars from that barrel.

Neanthes arenaceodentata

75. As with N. incisa, laboratory cultured juvenile Neanthes arenaceodentata were exposed to suspensions (≈ 200 mg/ℓ) of either REF or BRH sediment in combination with either a 100% REF or 100% BRH solid phase bedded sediment for 10 days.

76. In Experiment A and B (Table 14), histopathologic changes observed were only in the BRH(suspended)/BRH(solid phase) treatment. In both experiments, all the worms (100% incidence) from the BRH/BRH treatment showed a decrease of red-staining mucous secreting cells and an increase in basophilic mucous secreting cells. Five worms (45%) from the BRH/BRH treatment from the Experiment B had parapodia with extensive metaplasia of the epidermis and also some cyst formation. Two animals (18%) had extensive metaplasia of the epidermis with fibrosis underneath that extended into the coelomic cavity (Figures 11 and 12).

77. The production and secretion of mucous is an essential and important function in Neanthes. First, mucous facilitates the movement of this species on the sediment surface and functions as an adhesive in the construction of mucous-lined interconnecting burrows within the sediment. Therefore, if changes in mucous secreting cells were to cause a decrease in ability to secrete mucous, it would interfere with the worm's mobility and ability to construct the burrows which

provide habitat and protection from predation. The lack of the mucous lining may permit the worm to contact the sediment more directly, and this would be a possible explanation for the lesions seen on the parapodia of the BRH/BRH exposed worms. However, if changes in the mucous secreting cells were to cause an increase in mucous production, this could indicate the initiation of a defense mechanism. In this case, the worms would be producing more mucous as a reaction to contact with undesirable materials in the sediment.

78. With N. arenaceodentata, there were some changes in the pattern of burrowing seen at the sides of the dish among the treatments, but it was not as distinct as with Nephtys incisa. Generally, N. arenaceodentata did not burrow as deeply into the BRH solid phase bedded sediment treatments. This difference in burrowing could, as with N. incisa, explain why differences were seen in worms in the BRH/BRH treatment and not the other treatments with BRH sediment present.

79. The reproducibility of both the epidermal and mucous cell pathologies and frequency of responses was very good as indicated in the results summarized in Table 14. It is significant that the mucous cells system in the tube building polychaetes (Neanthes) responded similarly to the mucous tube glands in the tube building amphipods. In both species, 100% of the organisms were affected at the highest tested concentrations. Ecologically, both Neanthes and Ampelisca rely upon the mucous cell systems for the development of burrows or tubes that are essential for the organism's survival. Since Nephtys incisa is not a tube builder, its mucous secreting cells are not as well developed and histologically prominent as those in Neanthes.

Table 14

Histopathologic Findings in Neanthes arenaceodentata Exposed to Black Rock Harbor Sediment for 10 Days

Organs Examined	Percent Abnormalities			
	Experiment A			
	REF/REF N*=15	BRH/REF N=13	REF/BRH N=15	BRH/BRH N=12
Epidermis	0%	0%	0%	100%
Muscle	0%	0%	0%	0%
Intestine	0%	0%	0%	0%
Nervous system	0%	0%	0%	0%
Reproductive tract	0%	0%	0%	0%
Mucous cells	0%	0%	0%	100%
	Experiment B			
	REF/REF N=15	BRH/REF N=15	REF/BRH N=15	BRH/BRH N=11
Epidermis	0%	0%	0%	45%
Muscle	0%	0%	0%	0%
Intestine	0%	0%	0%	0%
Nervous system	0%	0%	0%	0%
Reproductive tract	0%	0%	0%	0%
Mucous cells	0%	0%	0%	100%

* N = number of animals examined.

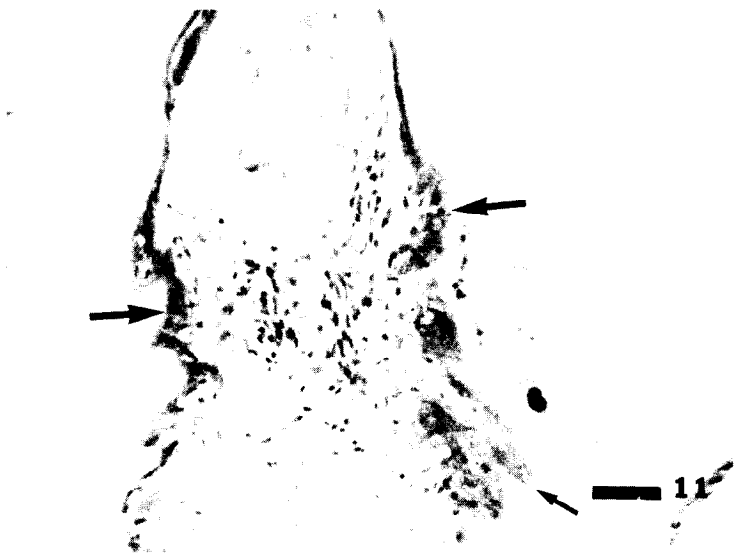

Figure 11. Parapodia of Neanthes exposed to BRH/BRH, Experiment B. Arrows point to metaplasia of the epidermis. Hematoxylin and eosin stain. Scale bar = 100 μm.

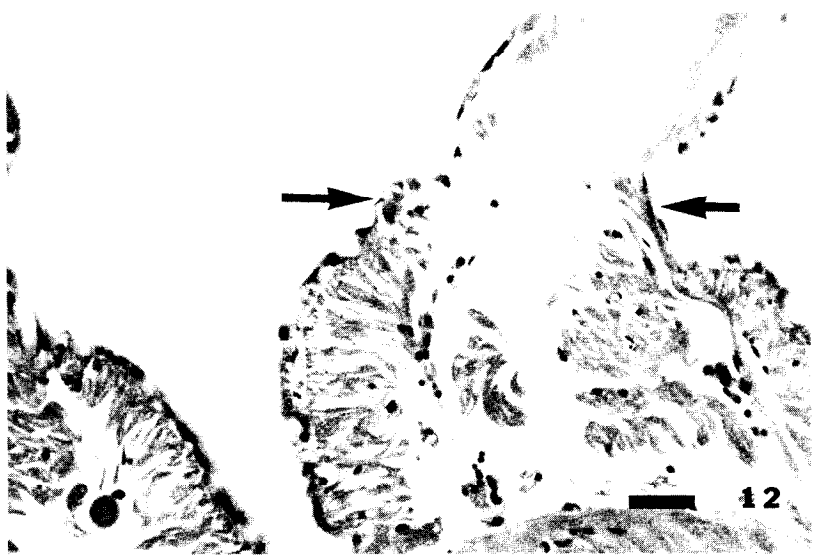

Figure 12. Parapodia of Neanthes exposed to BRH/REF, Experiment B. Arrows point to normal epidermis. Hematoxylin and eosin stain. Scale bar = 100 μm.

PART IV: CONCLUSIONS

80. Histopathological studies were conducted on the tissues and organ systems of several organisms exposed to Black Rock Harbor sediment. These studies were conducted to evaluate (a) the applicability and sensitivity of histopathological changes as measures of effects of dredged material contaminants, and (b) the degree of reproducibility and variability of this procedure. The organisms examined in this study include the filter feeding mollusc, Mytilus edulis; a deposit feeding mollusc, Yoldia limatula; the infaunal filter feeding crustacean, Ampelisca abdita; and the infaunal polychaete annelids, Nephtys incisa and Neanthes arenaceodentata.

81. The histopathological changes were detected in the female reproductive tract and the cardiovascular sytems of M. edulis exposed to suspended BRH sediments. Reproductive tract changes include basophilic ova that show a loss of nuclei, normal cytoplasm, and vitelline membrane. These changes probably resulted from the combination of spawning condition and BRHcontaminant stress. The cardiovascular effects were characterized by pedunculated growths arising from the auricle, ventricle, or pericardial walls. The sensitivity of the histopathological responses was similar to that reported for sublethal physiological measures. The degree of reproducibility of the histological changes was excellent considering the subjective nature of the endpoint and sample sizes examined.

82. There is a well defined pattern of histological changes in Ampelisca abdita exposed to BRH bedded and suspended sediments. Necrosis of gill epithelium and lamellae and loss of normal gill architecture were detected in all organisms exposed to high concentrations of BRH sediments.

Similarly, all animals exposed to high concentrations of BRH sediments had atrophied and vacuolated mucous cells and mucous tube glands as well as a loss of the basophilic staining granular material. The types of pathologies were similar whether bedded sediments or suspended sediments were the source of contamination. Pathologies were observed at exposure concentrations similar to those causing mortality and, while providing insight into the causes of mortality, did not provide an added measure of sensitivity. The reproducibility and variability were excellent in the definitive tests.

83. Yoldia limatula showed no histopathological changes under any of the experimental conditions studies. The lack of both histological effects and mortalities is surprising since the species is an infaunal deposit feeding bivalve mollusc that should have experienced maximal exposure. A possible explanation is that they did not actively feed and process sediment in the high BRH treatments and thus did not receive the expected exposures. This is supported by results of feeding studies (Rogerson et al., 1985) and coupled with the short duration of the test probably accounts for the absence of effects.

84. Histopathological changes were noted in the epidermis and muscle of both Nephtys incisa and Neanthes arenaceodentata. These changes were characterized by degeneration of parapodial muscles and metaplasia of the epidermis. In Neanthes, histological changes were reported for the mucous secreting cells in 100% of the organisms experiencing maximal exposure to BRH sediments. The secretion of mucous is essential for facilitating movement over sediment surfaces and as an adhesive for the construction of burrows. Interference with this function

will certainly have serious ecological implications for these species. It is interesting to note that similar histological changes in the mucous cell system were observed in the tube building amphipod.

85. The results of these studies lead to the following general conclusions. Histopathological changes can be used as measures of contaminant effects from dredged materials. The histological responses are no more sensitive than traditional measures of survival in short-term tests (< 10 days) and are of similar sensitivity to sublethal responses in longer term tests (30 days). The value of histopathology lies more in its interpretation and explanation of the causes of mortality or other observed sublethal responses. Considering the subjective nature of this subject area, it is surprising to find the high degree of reproducibility evident in this study. The strength of this approach lies more as an indication of long-term sublethal stress and as it provides supporting evidence and explanation for organismal level responses.

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GLOSSARY*

amebocytes:	types of blood cells found in invertebrates.
carcinogenic:	causing cancer.
degeneration:	a retrogressive pathologic change in cells or tissues in consequence of which the functions may be inhibited or destroyed.
depuration:	removal of waste products or environmental contaminants; purification.
fixative:	a substance used to preserve gross and histological specimens of tissue, usually by denaturing and precipitating or cross-linking the protein constituents.
histopathology:	the science dealing with the cytologic and histologic structure of abnormal or diseased tissue.
hyalin:	a clear eosinophilic, homogeneous substance occurring in degeneration.
mutagen:	any agent that causes the production of mutation.
metaplasia:	an abnormal change from one adult type of cell to another adult type.
necrosis:	the pathologic death of one or more cells or a portion of tissue or organ resulting from irreversible damage.
tumor:	an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissue.

* Sources: Stedman's Medical Dictionary
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